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(54) Title: PROCESS FOR RECOVERY OF A β-LACTAM ANTIBIOTIC

(57) Abstract

Process for recovery of a β-lactam antibiotic from a mixture containing β-lactam antibiotic and D-phenyl glycine (FG) in solution, with the mixture being brought to a pH between 3 and 8 at a temperature between −5 and 20 °C and at a concentration such that FG remains in solution, the solid β-lactam antibiotic obtained being recovered and the remaining liquid being subjected to a temperature increase to a temperature between 10 and 60 °C, with formation of solid FG, FG being separated out as a solid, and the mother liquor being at least partially recirculated. Use is preferably made of an initial mixture substantially containing β-lactam antibiotic and FG originating from an enzymatic acylation reaction in which the corresponding β-lactam nucleus, in particular 6-aminopenicillanic acid, 7-aminodesacetoxycephalosporanic acid and 7-amino-3-chloro-cet-3-em-4-carboxylic acid, is acylated with a D-phenyl glycine derivative.

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PROCESS FOR RECOVERY OF A β-LACTAM ANTIBIOTIC

The invention relates to a process for recovery of a β-lactam antibiotic from a mixture substantially containing β-lactam antibiotic and D-phenyl glycine (FG) in solution, with the mixture being brought to a pH between 3 and 8 at a temperature between -5 and 20°C and at a concentration such that FG remains in solution, the solid β-lactam antibiotic obtained being recovered and the remaining liquid being subjected to a temperature increase to a temperature between 10 and 60°C, with formation of solid FG, FG being separated out as a solid, and the mother liquor being recirculated.

In general, in the preparation of β-lactam antibiotics involving the acylation of a β-lactam nucleus with a derivative of D-phenyl glycine, the β-lactam antibiotic is difficult to recover and the reaction mixture is difficult to work up. Working up often involves substantial losses of valuable components, in particular the β-lactam antibiotic, partly in the form of solubility losses and partly because of degradation as a result of the limited stability of β-lactam antibiotics.

The invention provides a new concept for recovery of β-lactam antibiotics whereby, in a simple process that can be applied on an industrial scale, the losses of β-lactam antibiotics are strongly reduced and also valuable D-phenyl glycine is recovered. The

invention is based on the fact that it has been found that FG can be heavily supersaturated, at relatively low temperature, in the solution containing FG and the antibiotic and can remain so for a long time without FG precipitating or crystallizing out. In consequence, it is possible to selectively recover the β-lactam antibiotic at low temperature by isolating it, after crystallizing, through a pH shift which does not cause the FG to crystallize out. Furthermore, it has been found that increasing the temperature of the mother liquor causes FG to crystallize out rapidly whilst the β-lactam antibiotic does not crystallize out. In this manner, FG can be recovered selectively. In the aforementioned manner, complete separation between β-lactam antibiotic and FG can be effected.

During the acylation reaction of the lactam nucleus and a suitable acylation agent, in particular an enzymatic acylation reaction with for example an amide of D-phenyl glycine, for example D-phenyl glycine amide (FGA) or an ester of D-phenyl glycine, for example the methyl ester of D-phenyl glycine (FGM), the acylation agent hydrolyzes with the β-lactam antibiotic to form D-phenyl glycine (FG).

The mixtures obtained after an acylation reaction may contain, besides the β-lactam antibiotic and FG, for example as yet unconverted β-lactam nucleus and/or acylation agent, for example FGA or FGM. It has been found that the exact compositions of the mixtures that may be applied in the process according to the invention are not particularly critical. Mixtures that may suitably be applied in the process according to the invention are preferably mixtures containing 10-1500 mM, in particular 50-1000 mM, β-lactam antibiotic; 0-1500 mM, in particular 0-1000 mM FG, 0-1000 mM, in

particular 0-200 mM β-lactam nucleus and 0-1000, in particular 0-400 mM D-phenyl glycine derivative.

The mixture of β-lactam antibiotic and FG is brought to such concentration and pH that all components, in particular the components mentioned, are dissolved. To that end, the pH is preferably chosen to be low , for example between 0 and 3, in particular between 0.3 and 2. When the process is carried out on a large scale, a more or less continuous working-up process is preferably opted for. A continuous dissolving process allows a shorter residence time at relatively high or low pH. If desired, any solid components still present can be separated out by for example filtration or ultrafiltration.

According to the invention, the mixture, which may still contain solid β-lactam antibiotic, is first brought to a pH between 3 and 8, preferably between 5.5 and 8, in particular between 6.5 and 7, with measures being taken, for example adding water, to ensure that the concentration of the reactants, in particular FG, is such that the reactants, optionally with the exception of the β-lactam antibiotic, remain in solution whether or not supersaturated. The temperature is between -5 and 20°C, preferably between -3 and 15°C, in particular between 0 and 10°C. The temperature is kept relatively low, because it has surprisingly been found that FG can be heavily supersaturated under these condition, without FG precipitating.

Furthermore, it has been found that a temperature increase of the mother liquor remaining after separation of the β-lactam antibiotic causes FG to crystallize rapidly in the form of large crystals that can be filtered readily. The temperature is

increased to a value between 10 and 60°C, preferably to a value between 12 and 50°C, in particular between 15 and 40°C, in which process the pH of the mixture may in principle be varied in the range from 3 to 8. The pH preferably is between 5.5 and 8, in particular between 6.5 and 7.5.

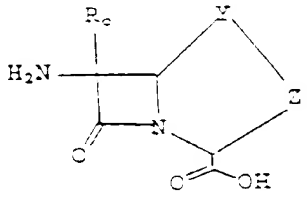
Now that the FG has crystallized out, the concentration has diminished so that, after being cooled, the mother liquor remaining after crystallization and separation of FG can at least partially be recirculated to for example the dissolving vessel, which also receives a fresh mixture of β-lactam antibiotic and FG. Such recirculation preferably takes place at such a rate that FG remains in solution in the dissolving vessel and remains supersaturated in the β-lactam antibiotic crystallization vessel. Since the FG mother liquor can at least partially be reused for β-lactam antibiotic crystallization, the solubility losses can be kept low. The degradation losses, too, are relatively low because of the favourable process conditions.

The process is preferably carried out continuously, with a fresh mixture of β-lactam antibiotic and FG being added all the time and a small proportion of for example the FG crystallization mother liquor being discharged all the time. Preferably, the flow of the discharge stream is chosen so that the volume of the process stream at various points in the process remains constant in time. In a continuous process, the discharge stream can in principle be smaller. A possible process scheme is given in Figure 1 by way of illustration.

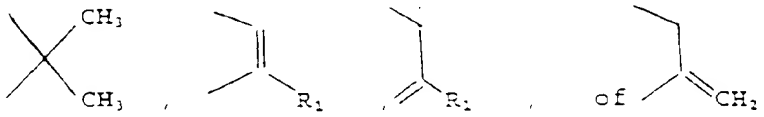
The process according to the invention can suitably be applied in the preparation of such β-lactam

antibiotics as have a phenyl glycine side chain, for example cefalexin, ampicillin, cefaclor, pivampicillin, becampicillin, talampicillin and cefaloglycine.

Any β-lactam nucleus can in principle be used, in particular a β-lactam nucleus with the general formula (1)



where R_6 represents H or an alkoxy group having 1-3 C atoms; Y represents CH_2, O, S or an oxidized form of sulphur; and Z represents



where R_1 represents for example H, OH, halogen, an alkoxy group having 1-5 C atoms, an alkyl group having 1-5 C atoms, a cycloalkyl group having 4-8 C atoms, an aryl or a heteroaryl group having 6-10 C atoms in which the groups may or may not be substituted with for example an alkyl, an aryl, a carboxy or an alkoxy group having 1-8 C atoms; and where the carboxylic acid group may be an ester group if so desired.

Suitable examples of β lactam nuclei that may be employed in the process according to the invention are penicillin derivatives, for example 6-aminopenicillanic acid (6-APA) and cephalosporanic acid derivatives, for example a 7-aminocephalosporanic acid with or without a substituent at the 3-site, for example 7-aminocephalosporanic acid (7-ACA), 7-

aminodesacetoxycephalosporanic acid (7-ADCA) and 7-amino-3-chloro-cef-3-em-4-carboxylic acid (7-ACCA).

In principle, any enzyme that is suitable as a catalyst in the coupling reaction can be used as the enzyme. Such enzymes include the enzymes collectively referred to as penicillin amidase or penicillin acylase. Such enzymes are described in for example J.G. Shewale et al., Process Biochemistry, August 1989, pp. 146-154 and in J.G. Shewale et all, Process Biochemistry International, June 1990, pp. 97-103. Examples of suitable enzymes are enzymes derived from Acetobacter, , in particular Acetobacter pasteurianum, Aeromonas, Alcaligenes, in particular Alcaligenes faecalis, Aphanocladium, Bacillus sp., in particular Bacillus megaterium, Cephalosporium, Escherichia, in particular Escherichia coli, Flavobacterium, Fusarium, in particular Fusarium oxysporum and Fusarium Solani, Kluyvera, Mycoplana, Protaminobacter, Proteus, in particular Proteus rettgari, Pseudomonas and Xanthomonas, in particular Xanthomonas citrii.

Preferably an immobilized enzyme is used, since the enzyme can be easily isolated and re-used then. A suitable immobilization technology is described for instance in EP-A-222462. Another suitable technology consists in immobilizing the Penicillin G acylase on a carrier which contains a gelating agent, for instance gelatin, and a polymer with free amino groups, for instance alginate amine, chitosan or polyethylene imine. In addition, enzymes may also be utilized as a crystalline substance (CLEC's™).

Particularly suitable enzymes among the immobilized enzymes that are commercially available are the Escherichia coli enzyme from Boehringer Mannheim GmbH, which is commercially available under the name

Enzygel®, the immobilized Penicillin-G acylase from Recordati and the immobilized Penicillin-G acylase from Pharma Biotechnology Hannover.

In the (enzymatic) acylation reaction, the acylation agent can be for instance a D-phenyl glycine in activated form, preferably a (primary, secondary or tertiary) amide or salt thereof, or a lower alkyl (1-4C) ester, for instance a methyl ester.

The temperature at which the enzymatic acylation reaction is effected usually is below 40°C, preferably between -5 and 35°C. The pH at which the enzymatic acylation reaction is effected usually is between 5.5 and 9.5, preferably between 6.0 and 9.0.

The reaction preferably is stopped almost completely when maximum conversion has been all but achieved. A suitable embodiment for stopping the reaction is to lower the pH, preferably to a value between 4.0 and 6.3, in particular between 4.5 and 5.7. Another suitable embodiment is to lower the temperature of the reaction mixture on attaining the maximum conversion. A combination of the two embodiments is possible also.

Once the reaction has been stopped on attaining the maximum conversion, the reaction mixture usually is present in the form of a suspension comprising a plurality of solids, for example the antibiotic, D-phenyl glycine and, possibly, immobilized enzyme. The immobilized enzyme preferably is recovered in the interest of process economics. This can suitably be accomplished for example by filtering the reaction mixture on a sieve, while stirring, the stirrer's direction of rotation being chosen so that the suspension is pumped upwards at the centre of the stirrer. Subsequently, valuable components such as the

antibiotic and FG can be recovered by the process according to the invention, with the solid components possibly apart from solid antibiotic, being dissolved first, by means of for example a pH shift.

The pH may be lowered in several ways in the framework of the invention, for instance by adding an acid to the mixture. Suitable acids are for example mineral acids, in particular sulphuric acid, hydrochloric acid or nitric acid. Preferably, hydrochloric acid is used. The pH can be raised by for example adding a base to the mixture. Suitable bases are for example inorganic bases, in particular ammonium hydroxide, potassium hydroxide or sodium hydroxide. Preferably, ammonium hydroxide is used.

In practice, the enzymatic acylation reaction and the working-up of the reaction mixture are usually effected in water. If desired, the reaction mixture may also contain an organic solvent or a mixture of organic solvents, preferably less than 30 vol.%. Examples of suitable organic solvents that can be used are alcohols having 1-7 C atoms, for example a monoalcohol, in particular methanol or ethanol; a diol, in particular ethylene glycol, or a triol, in particular glycerol.

The process according to the invention is particularly suited for being used in working up the reaction mixture obtained after the enzymatic acylation reaction in which 6-APA is acylated with an amide of D-phenyl glycine, for example FGA, or an ester of D-phenyl glycine, for example FGM.

In a preferred embodiment of the process measures are taken to ensure that the concentration of dissolved 6-APA in the reaction mixture is kept relatively low so that a higher conversion can be achieved than when the concentration of dissolved 6-APA

is chosen to be as high as possible.

Moreover, it has been found that the stirrability of the reaction mixture is significantly higher when the concentration of dissolved 6-APA is kept low.

In the present context, 'conversion' refers to the molar ratio of the ampicillin formed and the amount of 6-APA used. The concentration of dissolved 6-APA is expressed as the amount of 6-APA in moles per kg of the reaction mixture; the total concentration, dissolved and undissolved, of 6-APA is expressed as the amount of 6-APA plus ampicillin in moles per kg of the total reaction mixture; the total reaction mixture may contain, besides the solution, a number of solids, for example 6-APA, ampicillin, phenyl glycine and immobilized enzyme.

The molar ratio of acylation agent and 6-APA, i.e. the total amount of phenyl glycine derivative added, divided by the total amount of 6-APA added, expressed in moles, is preferably less than 2.5. It is preferred for the molar ratio to be between 1.0 and 2.0, in particular between 1.2 and 1.8.

The enzymatic acylation reaction is preferably carried out as a batch process. If desired, the reaction can also be carried out continuously, with in-line control of the concentration of dissolved 6-APA.

The total concentration of 6-APA plus ampicillin (in dissolved and undissolved form) in the reaction mixture preferably is higher than 250 mM, more preferably higher than 300 mM, in particular higher than 350 mM.

In this preferred embodiment, the concentration of dissolved 6-APA during the preparation of ampicillin is essentially kept below 300 mM.

preferably below 250 mM. At a higher concentration of the acylation agent, the concentration of dissolved 6-APA may optionally chosen to be higher than at a lower concentration. This is because the rate of reaction is higher at higher concentrations of the acylation agent, so that 6-APA is dissolved in a high concentration for only a relatively short period.

The concentration of 6-APA dissolved in the reaction mixture can be kept low in various ways. One possibility of keeping the concentration of dissolved 6-APA low is to initially feed only a portion of the total amount of 6-APA and to meter in the balance during the reaction. A drawback of this is that in that case 6-APA needs to be metered in solid form, which presents practical problems. Therefore, it is preferred in a batch process for the total amount of 6-APA to be supplied at the start of the reaction, whereupon, during the enzymatic acylation reaction, the concentration of 6-APA in the reaction mixture will decrease and the concentration of ampicillin will increase. A suitable method of achieving a low concentration of dissolved 6-APA is for example to keep the pH at a lower value than that at which maximum solubility of the reactants is achieved. A particularly suitable method of keeping the dissolved 6-APA concentration low is for example to ensure that the concentration of the phenyl glycine derivative is kept low, for example by metering in the phenyl glycine derivative partly in the course of the reaction.

In this context, it has been found that, if the concentration of the phenyl glycine derivative is kept low, only little 6-APA is dissolved so that the dissolved 6-APA concentration can be controlled by metering the phenyl glycine derivative.

A particularly suitable embodiment is

obtained when FGA is added in the form of one of its salts, preferably the salt of FGA and a mineral acid, for example FGA.HCl, FGA.$1/2H_2SO_4$ and FGA.HNO_3. In this manner it is possible to readily ensure optimum metering of the FGA by keeping the pH constant. Preferably, FGA.$1/2H_2SO_4$ is used inasmuch as this salt possesses extremely high solubility.

In the framework of the present invention the various components may be present in the reaction mixture in the free form or as salts. The pH values mentioned are in all cases the pH values measured at room temperature.

The invention will be further elucidated by means of the following examples, without however being restricted thereto.

Abbreviations:

AMPI = ampicillin
AMPI.$3H_2O$ = ampicillin trihydrate
6-APA = 6-amino-penicillanic acid
FGA = D-phenyl glycine amide
FG = D-phenyl glycine
FGHM = D-p-hydroxyphenyl glycine methyl ester

Assemblase™ is an immobilized Escherichia coli penicillin acylase from E. coli ATCC 1105 as described in WO-A-97/04086. The immobilization is effected as set out in EP-A-222462, with gelatin and chitosan being used as gelating agents and glutaraldehyde as crosslinking agent.

The ultimate activity of the Escherichia coli penicillin acylase is determined by the amount of enzyme added to the activated spherules and amounted to 3 ASU/g of dry weight, 1 ASU (Amoxicillin Syhthesis Unit) being defined as the amount of enzyme capable of

producing 1 g of Amoxicillin.$3H_2O$ from 6-APA and FGHM per hour (at 20°C; 6.5% 6-APA and 6.5% FGHM).

Example I

Preparation of FGA.$1/H_2SO_4$ solution

301.6 g of FGA (2.00 mole) were suspended in 650 g of water at T = 5°C. 102.1 g of 96-% H_2SO_4 (1.00 mole) were added drop-wise in 1 hour while stirring, with the temperature being kept at T < 25°C by means of cooling.

Example II

Enzymatic synthesis of ampicillin

An enzyme reactor (1.5 l, diameter 11 cm), fitted with a 175 µm mesh sieve bottom, was filled with 300 g of net-wet assemblase™ (the term net-wet refers to the mass of the enzyme obtained on separating the enzyme from an enzyme slurry with the aid of a glass filter).

A preparation reactor (1.2 l) was filled with 131.6 g of 6-APA (0.600 mole), 30.2 g of FGA ((0.200 mole) and 400 ml of water (T = 10°C). This mixture was stirred for 15 minutes at T = 10°C and then at t = 0 transferred into the enzyme reactor with the aid of 100 ml of water (T = 10°C).

The stirrer in the enzyme reactor was switched on at t = 0. The temperature was kept at 10°C all the time. 423.7 g of FGA.$\frac{1}{2}H_2SO_4$ solution (0.800 mole) were added at a constant rate over a period of 233 minutes. The pH was approx. 6.3. From t = 295 minutes onwards the pH was maintained at 6.3 by titrating with 6N H_2SO_4. At t = 570 minutes the amount of AMPI was maximum and the pH was reduced to 4.7 by adding 6N H_2SO_4. The enzyme

reactor now contained:
575 mmole AMPI
15 mmole 6-APA
50 mmole FGA
365 mmole FG

Example III

Separation of AMPI/FG slurry from enzyme reactor

The AMPI/FG slurry prepared as described in Example II was removed from the enzyme reactor via the sieve bottom by means of stirred filtration. This was done using a pitched-blade stirrer, which was positioned at 0.5 cm over the sieve. Stirring was in upward direction at approx. 500 rpm. The AMPI/FG slurry separated from the reactor was filtered on a G3 glass filter. The AMPI/FG wet cake was put aside and the mother liquor was returned to the enzyme reactor, whereupon stirred filtration followed by G3 filtration of the AMPI/FG slurry resumed. The enzyme reactor was washed with the AMPI/FG mother liquor in this fashion until no more solid matter was flushed out of the reactor. The last mother liquor collected in G3 filtration was combined with the AMPI/FG wet cake to form an AMPI/FG slurry.

The AMPI/EG slurry so obtained contained > 99.0% of the total amount of AMPI produced in the enzyme reactor and > 95.5% of the total amount of FG produced in the enzyme reactor. After this stirred filtration, > 99.5% of the Assemblase™ was present in the enzyme reactor.

Example IV

Recrystallization of ampicillin

Recrystallization was effected in a rig (Fig. 1) consisting of a storage vessel (V_1; 2 l), a

pump, a dissolving vessel (V_1; 0.05 l), a filter (F_0) fitted with a Seitz filter plate, a pump, an AMPI crystallization vessel (V_3; 0.5 l), two glass filters 1A and 1B (F_{1A} and F_{1B}) arranged in parallel, a pump, a heat exchanger, an FG crystallization vessel (V_4; 0.5 l), two glass filters 2A and 2B (F_{2A} and F_{2B}) arranged in parallel, a pump and lastly a heat exchanger connected to the dissolving vessel. The line between filters 2A and 2B and the dissolving vessel contained a three-way valve enabling a portion of the stream to be discharged. All vessels were provided with a stirrer, a thermometer and a pH electrode.

The AMPI/FG slurry which was isolated as described in Example III was quantitatively transferred to the storage vessel and cooled to 2°C while being stirred.

The recrystallization loop (from the storage vessel up to the heat exchanger inclusive) was filled with a total of approx. 1350 grams of initial solution consisting of an aqueous solution of 0.6% AMPI and 0.6% FG. The recrystallization loop (except the FG crystallization vessel and the glass filters 2A and 2B) was cooled to 1-2°C. The FG crystallization vessel and the glass filters 2A and 2B were kept at 20°C. Circulation in the recrystallization loop (flow = 1.2 litre per hour) was started. The pH in the dissolving vessel was adjusted to pH = 1.25 by titration with 8N HCl solution. The pH in the AMPI crystallization vessel was adjusted to pH = 6.5 by titration with 25 wt.% NH_3 solution. 13.0 grams of AMPI.$3H_2O$ were added to the AMPI crystallization vessel as nuclei. 10.0 grams of FG nuclei were added to the FG crystallization vessel.

Recrystallization was started at t = 0 by

metering the AMPI/FG slurry from the storage vessel to the dissolving vessel (flow = 0.14 litre per hour). The contents of the storage vessel were added to the dissolving vessel in approx. 8 hours. The levels in the dissolving vessel and the AMPI and FG crystallization vessels remained constant throughout. This was accomplished by discharging a proportion of the FG mother liquor to the dissolving vessel rather than recirculating it.

The AMPI slurry from the AMPI crystallization vessel was filtered on glass filter 1A without interruption while the mother liquor was being pumped to the FG crystallization vessel. The FG slurry from the FG crystallization vessel was filtered on glass filter 2A and the mother liquor was pumped back to the storage vessel. The AMPI/FG slurry from the storage vessel and the FG mother liquor were mixed in the dissolving vessel at a ratio of 1 to 8.6 throughout the experiment. The storage vessel was empty after approx. 8 hours and a total of 330 ml of 8N HCl solution had been metered into the dissolving vessel.

At t = 8 hours the storage vessel was filled with fresh AMPI/FG slurry that had been isolated as described in Example III. AMPI separation in the recrystallization loop was switched from glass filter 1A to 1B and FG separation was similarly switched from glass filter 2A to 2B.

The AMPI wet cake on glass filter F1A was washed with 2 x 175 ml of water (T = 5°C) and dried. The FG wet cake on glass filter 2A was washed with 2 x 60 ml of water and dried.

Recrystallization was carried out continuously by filling the storage vessel with AMPI/FG slurry every 8 hours and by alternately inserting and

emptying the glass filters 1A and 1B and 2A and 2B. The flows of FG and mother liquor were approx. 0.18 litre per hour on average.

The average yield per charge in the storage vessel (600 mmoles of 6-APA metered into the enzyme reactor in Example II) amounted to 220 grams of AMPI.$3H_2O$ (exclusive of AMPI nuclei; 91% relative to 600 mM of 6-APA) and 30 grams of FG (exclusive of FG nuclei).

C L A I M S

1. Process for recovery of a β-lactam antibiotic from a mixture containing the β-lactam antibiotic and D-phenyl glycine (FG) in solution, with the mixture being brought to a pH between 3 and 8 at a temperature between -5 and 20°C and at a concentration such that FG remains in solution, the solid β-lactam antibiotic obtained being recovered and the remaining liquid being subjected to a temperature increase to a temperature between 10 and 60°C, with formation of solid FG, FG being separated out as a solid, and the mother liquor being at least partially recirculated.
2. Process according to Claim 1, in which the temperature of the mixture is between -3 and 15°C.
3. Process according to Claim 2, in which the temperature of the mixture is between 0 and 10°C.
4. Process according to any one of Claims 1-3, in which the temperature in increased to a value between 12 and 50°C.
5. Process according to Claim 4, in which the temperature is increased to a value between 15 and 40°C.
6. Process according to any one of Claims 1-5, in which the mixture is brought to a pH between 4 and 7.
7. Process according to any one of Claims 1-6, in which the mother liquor is recirculated to the mixture containing dissolved β-lactam antibiotic and FG.
8. Process according to any one of Claims 1-7,

characterized in that the process is carried out continuously.

9. Process according to any one of Claims 1-8, in which the initial mixture substantially containing β-lactam antibiotic and FG originates from an enzymatic acylation reaction in which the corresponding β-lactam nucleus is acylated with a D-phenyl glycine derivative.

10. Process according to Claim 9 in which the mixture obtained after the acylation reaction is first subjected to a pH reduction to a pH between 0 and 3.

11. Process according to Claim 10 in which the mixture is subjected to a pH reduction to a pH between 0.3 and 2.

12. Process according to any one of Claims 1-11 in which the mixture contains 10-1500 mM β-lactam antibiotic, 0-1500 mM FG, 0-1000 mM D-phenyl glycine derivative and 0-1000 mM β-lactam nucleus.

13. Process according to any one of Claims 1-12 in which the β-lactam nucleus is 6-aminopenicillanic acid (6-APA) and the β-lactam antibiotic is ampicillin.

14. Process according to any one of Claims 1-12 in which the lactam nucleus is 7-aminodesacetoxycephalosporanic acid (7-ADCA) and the β-lactam antibiotic is cephalexin.

15 Process according to any one of Claims 1-12 in which the lactam nucleus is 7-amino-3-chloro-cef-3-em-4-carboxylic acid (7-ACCA) and the β-lactam antibiotic is cefaclor.

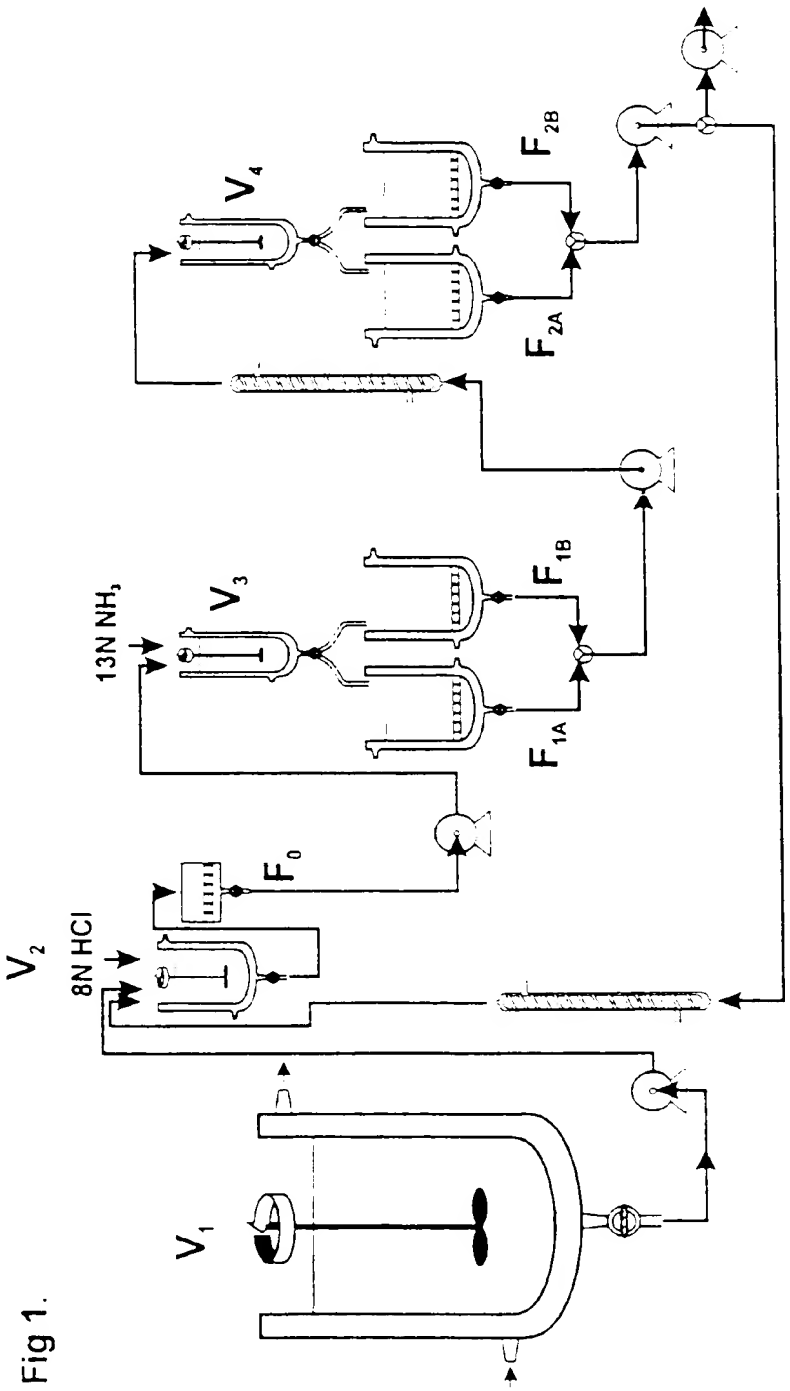


Fig 1.

INTERNATIONAL SEARCH REPORT

Int ional Application No
PCT/NL 98/00539

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C07C409/18 C07C501/12 C12P37/04 C12P35/04

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C07C C12P A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	WO 97 22610 A (CHEMFERM V.O.F.) 26 June 1997 see page 3, line 7 - page 4, line 19; claims	1-15
A	WO 96 30376 A (CHEMFERM V.O.F.) 3 October 1996 see the whole document	1-15
A	WO 96 23796 A (CHEMFERM V.O.F.) 8 August 1996 see the whole document	1-15
A	WO 95 03420 A (DSM N.V.) 2 February 1995 see the whole document	1-15

-/--

[X] Further documents are listed in the continuation of box C

[X] Patent family members are listed in annex

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
24 November 1998	01/12/1998

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl Fax (+31-70) 340-3016	Chouly, J

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INTERNATIONAL SEARCH REPORT

Int. ional Application No
PCT/NL 98/00539

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No
A	US 5 034 522 A (F. G. SCHREIBER) 23 July 1991 see claims	1-15
P,A	NL 1 002 818 C (CHEMFERM V.O.F.) 15 October 1997 see claims	1-15

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INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte ...al Application No
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